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Variability in Response to Quadripulse Stimulation of the Motor Cortex

Koichiro Nakamura [a], Stefan Jun Groiss [a,b], Masashi Hamada [c], Hiroyuki Enomoto [a], Suguru Kadowaki [a], Mitsunari Abe [a], Takenobu Murakami [a], Winnugroho Wiratman [a], Fangyu Chang [a], Shunsuke Kobayashi [a], Ritsuko Hanajima [d], Yasuo Terao [c], Yoshikazu Ugawa [a,e,*]

[a] Department of Neurology, School of Medicine, Fukushima Medical University, Fukushima, Japan
[b] Department of Neurology, Center for Movement Disorders and Neuromodulation, Institute of Clinical Neuroscience, Medical Psychology Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany
[c] Department of Neurology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
[d] Department of Neurology, School of Medicine, Kitasato University, Sagamihara, Japan
[e] Fukushima Global Medical Science Center, Advanced Clinical Research Center, Fukushima Medical University

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ABSTRACT

Background: Responses to plasticity-inducing brain stimulation protocols are highly variable. However, no data are available concerning the variability of responses to quadripulse stimulation (QPS).
Objective: We assessed the QPS parameters of motor cortical plasticity induction in a systematic manner, and later investigated the variability of QPS using optimal parameters.
Methods: First, two different interburst intervals (IBI) with the same total number of pulses were compared. Next we investigated three different IBIs with a different total number of pulses but with same duration of intervention. We also compared the after-effects of monophasic and biphasic QPS. Finally, variability of QPS was tested in 35 healthy subjects. Twenty motor evoked potentials (MEPs) were measured every 5–10 min for up to one hour after intervention.
Results: QPS at an IBI of 5 s produced MEPs changes that are dependent on the interstimulus interval of the four magnetic pulses, consistent with previous reports. Unexpectedly, QPS at an IBI of 2.5 s did not induce any plasticity, even with the same total number of pulses, that is, 1440. QPS at an IBI of 7.5 s produced a variable response but was likely to be comparable to conventional QPS. Biphasic QPS had shorter lasting after-effects compared with monophasic QPS. Finally, the after-effects of QPS were relatively consistent across subjects: more than 80% of subjects responded as expected in the excitatory QPS at an IBI of 5 s.
Conclusions: The IBI, total duration of the procedure and pulse waveform strongly affected the magnitude or duration of the plasticity induced by QPS. In this cohort, 80% of subjects responded to excitatory QPS as expected.

Introduction

Non-invasive brain stimulation (NIBS) has been widely used in a variety of neuroscience fields and clinical settings. This is primarily due to its ability to induce lasting after-effects after the stimulation period. Indirect evidence in which N-Methyl-D-Aspartate (NMDA) receptor antagonist blocks at least some of the effects induced by NIBS suggests that they might represent an analog of synaptic plasticity in the human brain [1]. Thus, NIBS offers a potential means for interfering with neuronal function, as well as therapeutic applications. Nonetheless, one of the major issues of any NIBS protocols is the high variability of their effects [2]. Given the high inter-individual variability in response to other plasticity protocols such as paired associative stimulation (PAS), theta-burst stimulation (TBS), and transcranial direct current stimulation (TDCS) in which 30%–50% of participants fail to respond in the "canonical" way [3–12], we aimed to investigate the variation in response to quadripulse stimulation (QPS), another NIBS protocol for plasticity induction [13–16].

* Corresponding author. Fax: +81 24 548 3797.
E-mail address: ugawa-tky@umin.net (Y. Ugawa).

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QPS consists of bursts of four monophasic TMS pulses, separated by inter-stimulus intervals (ISI) of 1.5, 5, 10, 30, 50 or 100 ms, which are repeated with an inter-burst interval (IBI) of 5 s (i.e., 0.2 Hz) for 30 min (i.e., 1440 pulses in total). Depending on the ISI, QPS induces bidirectional changes of corticospinal excitability as indexed by the size of the motor evoked potential (MEPs); QPS at short ISIs (QPS-1.5, QPS-5, QPS-10) produces a long-lasting increase in MEP, while QPS at long ISIs (QPS-30, QPS-50, and QPS-100) induces a lasting MEP decrease for approximately one hour [14]. In the original report, the stimulus intensity and duration of QPS that is proportional to the total number of pulses are systematically investigated; there was no difference in the amount and duration of plasticity when using a QPS intensity of approximately 130% of active motor threshold (AMT) (suprathreshold) and 90% AMT (subthreshold) [13]. For the duration of QPS, 30 min (i.e. 1440 pulses) but not 15 minutes (i.e., 720 pulses) was sufficient to induce facilitation of MEPs. However, the optimal IBI and total number of pulses (or duration of QPS) for inducing the largest after-effect remain unclear. We hypothesized that the total duration of monophasic QPS (i.e., 30 min) can be shortened to 15 min when an IBI of 2.5 s is used for monophasic QPS because the total number of pulses of this particular protocol is identical to conventional QPS (i.e., 1440 pulses). However, this result did not support our hypothesis due to the lack of plasticity by QPS at an IBI of 2.5 s. Thus, we subsequently assessed these parameters in a systematic manner although we were not able to study them fully due to mutual interaction between certain parameters. Furthermore, we hypothesized that QPS using monophasic pulses may be more effective than conventional rTMS using biphasic pulses because monophasic repetitive TMS (rTMS) is likely to be more powerful than biphasic rTMS [17]. This hypothesis was also assessed by comparison between monophasic QPS and biphasic QPS in this investigation.

Finally, we investigated the variability of QPS using optimal parameters. We considered that this variability had practical importance in clarifying these issues, given the potential uses of QPS in clinical settings.

Materials and methods

Subjects

Thirty-five right-handed subjects (8 females, 27 males; 20–53 years old, mean age ± SD: 37.7 ± 8.4) participated in this study. None of the participants had any contraindications to TMS, took any medication on a regular basis or had a positive history of psychiatric or neurologic diseases [18]. All subjects provided written informed consent to participate in this study. This study was approved by the Ethics Committee of Fukushima Medical University and the University of Tokyo.

Recordings

During the experiment, subjects were seated on a comfortable chair. The right first dorsal interosseus (FDI) muscle activity was recorded via Ag/AgCl cup electrodes in a belly-tendon montage. Raw signals were amplified and bandpass filtered (100 Hz–3 kHz, Multi Amplifier 1000, DIGITEX LAB Co. Ltd., Japan). Signals were digitized at 5 kHz and data were stored on a computer for offline analysis (MultiStim tracer; Medical Try System, Japan).

Transcranial magnetic stimulation

Single-pulse TMS was performed with a figure-of-eight coil (internal wing diameter of 7 cm) connected to a Magstim 200^2 stimulator (The Magstim Co. Ltd). The hotspot was identified as the position where the largest motor evoked potential (MEPs) were elicited when applying the same intensity stimulation with the coil held 45 degrees to the midline, tangentially to the skull and the handle pointing backwards. The spot was consecutively marked on the scalp with a waterproof pen alongside with 2 additional orientation marks needed for exact repositioning of the coil. The resting motor threshold (RMT) was determined as minimum stimulator output intensity needed to achieve a minimum MEP-amplitude of 50 μV in the completely relaxed FDI-muscle in at least 5 out of 10 trials. We also assessed active motor threshold (AMT) as the lowest stimulator output intensity evoking an MEP of at least 200 μV in 5 out of 10 consecutive trials while subjects maintained 10% of their maximum voluntary contraction (MVC) in the target muscle.

Quadripulse stimulation (QPS)

Quadripulse stimulation (QPS) was applied to the hand area of the left motor cortex using a combining module (The Magstim Co. Ltd.) connected with four stimulators (Magstim 200^2, The Magstim Co. Ltd.) as previously reported [14]. The conventional QPS protocol consisted of a burst of four TMS pulses separated by an interburst interval (IBI) of 5 s. Each burst consisted of four monophasic magnetic pulses separated by interstimulus intervals (ISI) of 5 ms (QPS5, excitatory QPS) or 50 ms (QPS50, inhibitory QPS), which can induce a potentiation and depression after-effect lasting up to 90 min [14]. The stimulus intensity of QPS was set at 95% AMT in all experiments. For experiment 1 (see below), we used the octopulse stimulation (OPS) system (The Magstim Co. Ltd.). It consists of eight monophasic magnetic stimulators (Magstim 200^2, The Magstim Co. Ltd.) connected with a specially designed combining module (The Magstim Co. Ltd). This device combines the outputs from eight stimulators to allow a train of eight monophasic magnetic pulses to be delivered through a single coil. Because it takes approximately 4 s for recharging one monophasic stimulator, the conventional QPS system is not able to accomplish QPS with an IBI of 2.5 s. We used two QPS systems connecting with the same coil through an octopulse system for giving QPS with an IBI of 2.5 s. For experiment 3, we used biphasic mode of QPS: it consists of four biphasic magnetic pulses produced by biphasic stimulators (Magstim SuperRapid, The Magstim Co. Ltd.) connected with a combining module (The Magstim Co. Ltd), which allows a train of four biphasic pulses to be delivered via a single coil.

Experimental parameters

As a measure of corticospinal excitability, we recorded twenty MEPs elicited by single pulse TMS with the intensity adjusted to evoke an approximately 0.5 mV peak-to-peak amplitude ($SI_{0.5\,mV}$) at rest at baseline condition. The stimulation intensity was kept constant throughout the experiment. The inter-trial interval was set at 4.5–5.5 s for the follow-up MEP measurements. Muscle activity was monitored throughout experiments using high gain audiovisual feedback (1000 uV/div) on an oscilloscope, which enabled them to keep their target muscles relaxed.

Study design (Fig. 1)

In all experiments, baseline corticospinal excitability measurements were followed by different QPS intervention (see below). After the application of QPS, 20 MEPs were recorded every 5 min for 30 min, and every 10 min from 30 to 60 min after the intervention (6–9 time points, T5, T10, T15, T20, T25, T30, T40, T50, T60) (see below).

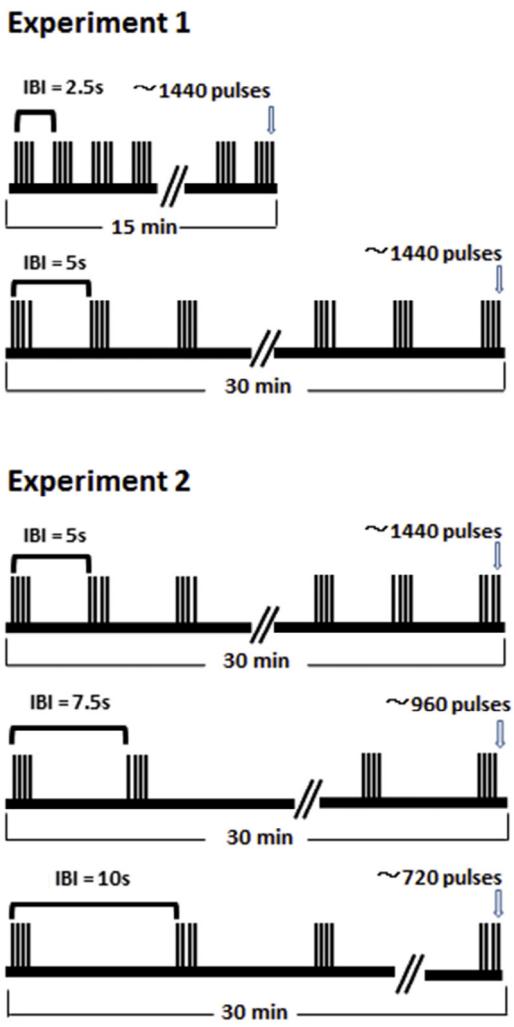

Figure 1. Original QPS protocol consists of 360 bursts of TMS pulses for 30 min with an interburst interval (IBI) of 5 s. Each burst consists of four monophasic magnetic pulses (i.e. quadripulse stimulation: QPS) delivered at inter-stimulus intervals (ISI) of 5 or 50 ms. In experiment 1, two protocols were tested, termed QPS-IBI2.5 (top row) and QPS-IBI5 (second row). In QPS-IBI2.5, IBI was set at 2.5 s, and total duration of QPS was 15 min, thus, in total 1440 pulses were induced. The other protocol was original QPS, which is equal to QPS-IBI5. In experiment 2, three conditions were tested, as QPS-IBI5 (third row), QPS-IBI7.5 (fourth row), and QPS-IBI10 (bottom row). As indicated, the total numbers of pulses were different because we fixed the total duration of QPS at 30 min.

Experiment 1

This experiment was performed to compare two different IBIs, 2.5 s vs. 5 s (Fig. 1). This experiment also allowed the study of the difference between total duration of QPS, 15 min vs. 30 min with the same total number of QPS pulses (i.e., 1440 pulses). The stimulus intensity of QPS was set at 95% AMT. Ten subjects participated in this experiment. Two IBIs were tested with excitatory QPS5 (n = 5) (QPS5-IBI2.5 vs. QPS5-IBI5) and inhibitory QPS50 (n = 5) (QPS50-IBI2.5 vs. QPS50-IBI5). MEP measurements were performed up to one hour after the end of QPS.

Experiment 2

We compared three different IBIs, 5, 7.5, and 10 s (Fig. 1). Because the total duration of QPS was fixed at 30 min, the total number of pulses was different among the conditions; 1440 pulses for an IBI of 5 s, 960 pulses for an IBI of 7.5 s, and 720 pulses for an IBI of 10 s. Ten subjects participated in this experiment. Three IBIs were tested with excitatory QPS5 (n = 5) (QPS5-IBI5, QPS5-IBI7.5, vs. QPS5-IBI10) and inhibitory QPS50 (n = 5) (QPS50-IBI5, QPS50-IBI7.5, vs. QPS50-IBI10). MEP measurements were performed up to an hour after QPS application.

Experiment 3

Monophasic and biphasic QPSs were compared. For biphasic QPS, the coil orientation was the same as in monophasic QPS condition. Stimulus intensity was set at 95% AMT, as measured using the biphasic QPS system. Twelve subjects participated in this experiment. Two configurations were tested with excitatory QPS5 (n = 6) (QPS5-Mono vs. QPS5-Bi) and inhibitory QPS50 (n = 6) (QPS50-Mono vs. QPS50-Bi). MEP measurements were performed up to an hour after the end of QPS.

Experiment 4

The experiment was performed to confirm the variability of the lasting effect of QPS. In total, 35 subjects participated (18 subjects were naïve). All subjects were enrolled in the following two experiments in a randomized order. Conventional excitatory QPS5 and inhibitory QPS50 were used in this experiment: QPS5 and QPS50 consist of bursts of four stimuli (i.e. an ISI of 5 ms or 50 ms), repeated every 5 s ((i.e. an IBI of 5 s) for 30 min (total 1440 pulses). The stimulus intensity was set at 95% AMT. MEP measurements were performed up to 60 min after QPS application.

In all of the above experiments, two consecutive experiments were separated by at least one week in the same subject.

Statistical analysis

In experiments 1 and 2, three-way repeated measures analysis of variance (ANOVA) was computed with factors ISI (QPS5 and QPS50), TIME (T5-T60) and IBI (experiment 1, 2.5 vs 5 s; experiment 2, 5 vs.7.5 vs. 10 s) using normalized MEP values after the intervention without including the baseline value (1.0). For experiment 3, three-way ANOVA using normalized MEP values after the intervention was performed with factors ISI (QPS5 and QPS50), TIME (T5-T30) and TYPE (monophasic vs. biphasic). The Greenhouse–Geisser correction was used if necessary to correct non-sphericity. For each QPS condition, one-way repeated measures ANOVA was applied with the factor TIME (baseline, T5-T60 for experiments 1 and 2; baseline, T5-T30 for experiment 4) using non-normalized MEP-values to confirm the significant changes from baseline MEP sizes. For baseline measurements data were reported as the mean value ± standard deviation (SD). Data were analyzed using SPSS-software (SPSS ver. 17.0 for Windows; SPSS Inc.).

Results

No adverse effects were reported. Baseline physiological measurements are shown in Table 1 and did not differ significantly between stimulation conditions.

Experiment 1

This experiment was performed to compare two different IBIs, 2.5 s vs. 5 s with the same total number of pulses (i.e., 1440 pulses) (Fig. 1). Thus, the difference between QPS-IBI2.5 and QPS-IBI5 is IBI as well as the total duration of QPS, 15 min vs. 30 min. Fig. 2A and B shows the time courses of QPS in each condition (Fig. 2A, excitatory QPS5; Fig. 2B, inhibitory QPS50). Consistent with previous reports [14], conventional excitatory QPS5-IBI5 and inhibitory QPS50-IBI5 (i.e. an IBI of 5 s, for 1440 pulses, Fig. 1) produced a substantial increase/decrease in corticospinal excitability for about one hour, whereas no changes were observed in corticospinal excitability after

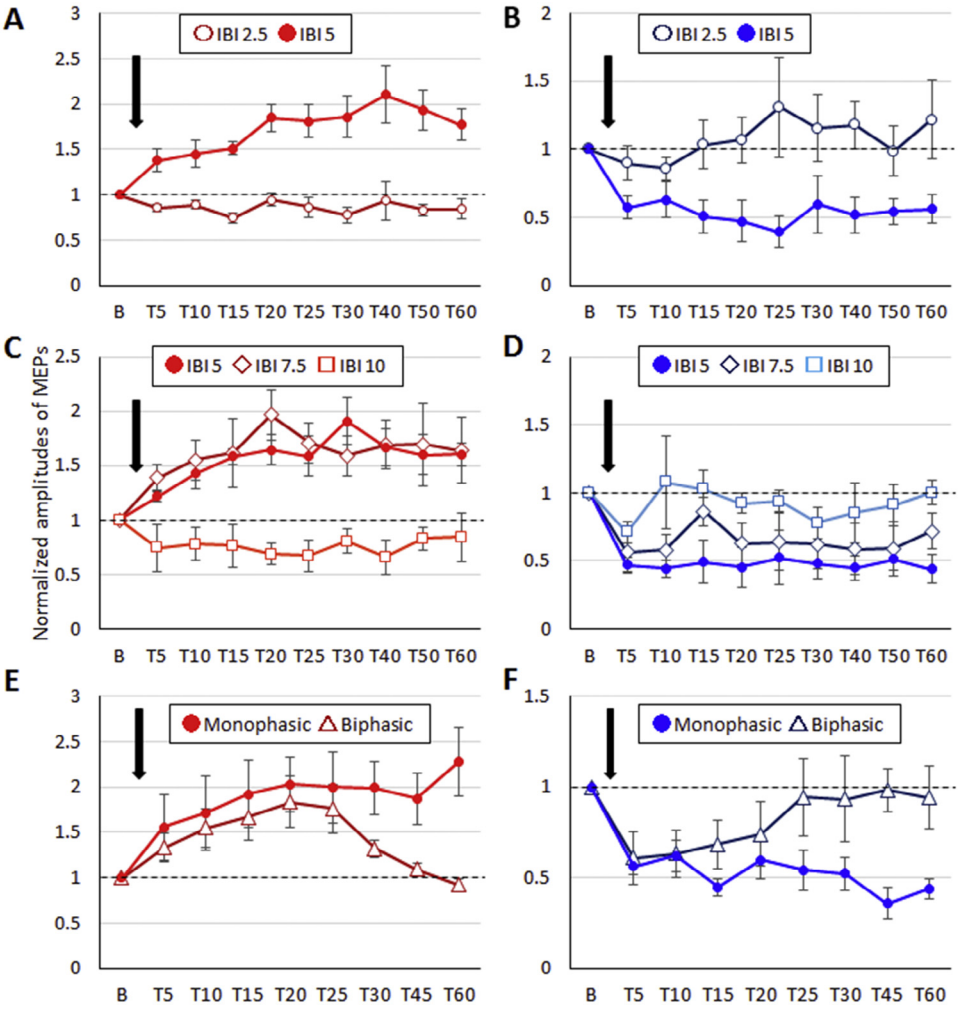

Figure 2. (A) Time courses of excitatory QPS5-IBI2.5 (red circle) and excitatory QPS5-IBI5 (filled red circle). Excitatory QPS5-IBI5 induced MEP facilitation, while no changes in MEP sizes after excitatory QPS5-IBI2.5. (B) Time courses of inhibitory QPS50-IBI2.5 (blue circle) and inhibitory QPS50-IBI5 (filled blue circle). Inhibitory QPS50-IBI5 induced MEP suppression, while no changes in MEP sizes after inhibitory QPS50-IBI2.5. (C) Time courses of excitatory QPS5-IBI5 (red filled circle), excitatory QPS5-IBI7.5 (red diamond), and excitatory QPS5-IBI10 (red square). Excitatory QPS5-IBI5 and excitatory QPS5-IBI7.5 had a similar time course, although variable responses were obtained by excitatory QPS5-IBI7.5. No changes were found in MEP sizes after excitatory QPS5-IBI10. (D) Time course of inhibitory QPS50-IBI5 (blue filled circle), inhibitory QPS50-IBI7.5 (blue diamond), and inhibitory QPS5-IBI10 (blue square). Inhibitory QPS50-IBI5 produced stable decrease of MEP sizes, but much less by inhibitory QPS50-IBI7.5 and inhibitory QPS50-IBI10. Error bars indicate the standard error. (E, F) Time course of excitatory QPS5-monophasic (red filled circle), excitatory QPS5-biphasic (red triangle), inhibitory QPS50-monophasic (blue filled circle), and inhibitory QPS50-biphasic (blue triangle). Biphasic QPS had a shorter duration effect than monophasic QPS. Error bars indicate standard error. In all figures, the black arrow indicates the timing of QPS. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

QPS5-IBI2.5 and QPS50-IBI2.5. Indeed, three-way repeated measures ANOVA on combined QPS5-IBI2.5/QPS5-IBI5/QPS50-IBI2.5/QPS50-IBI5 data revealed significant main effects of ISI, ISI × IBI interaction, but no effects of TIME, IBI, IBI × TIME, ISI × TIME, nor ISI × IBI × TIME interactions (Table 2). Post hoc analysis showed a significant difference between QPS5-IBI2.5 and QPS5-IBI5 (Bonferroni corrected, $P < 0.001$), and between QPS50-IBI2.5 and QPS50-IBI5 (Bonferroni corrected, $P = 0.033$). One-way ANOVA on excitatory QPS5-IBI5 and inhibitory QPS50-IBI5 data separately revealed a significant main effect of TIME, while excitatory QPS5-IBI2.5 and inhibitory QPS50-IBI2.5 did not reveal an effect (Table 3). Any interventions using IBI2.5 induced no motor cortical excitability changes.

Experiment 2

This experiment was performed to compare three different IBIs, 5 s, 7.5 s, vs. 10 s with the same total duration of QPS (i.e., 30 min), and thus the total pulses of QPS were different among the protocols; 1440 pulses for QPS-IBI5, 960 pulses for QPS-IBI7.5, and 720 pulses for QPS-IBI10 (Fig. 1). Fig. 2C and D shows the time courses of each condition. There were clear MEP changes after QPS-IBI5, whereas no changes after QPS-IBI10. For the QPS-IBI7.5, it is likely that there are certain changes in MEP sizes, but these changes were less stable than QPS-IBI5. Three-way repeated measures ANOVA revealed significant main effects of ISI and ISI × IBI interaction, but no effects of TIME, IBI × TIME, ISI × TIME, ISI × IBI × TIME interactions (Table 2). Post hoc analysis revealed significant difference between excitatory QPS5-IBI5 and excitatory QPS5-IBI10 (Bonferroni corrected, $P = 0.003$), excitatory QPS5-IBI7.5 and excitatory QPS5-IBI10 (Bonferroni corrected, $P = 0.002$), inhibitory QPS50-IBI5 and inhibitory QPS50-IBI10 (Bonferroni corrected, $P = 0.031$), but no difference between excitatory QPS5-IBI5 and excitatory QPS5-IBI7.5 (Bonferroni corrected, $P = 1.000$), inhibitory QPS50-IBI5 and inhibitory QPS50-IBI7.5 (Bonferroni corrected, $P = 0.792$), inhibitory QPS50-IBI7.5 and inhibitory QPS50-IBI10 (Bonferroni corrected, $P = 0.261$),

Table 1
Baseline physiological data.

	RMT	AMT	AMT biphasic	Baseline MEP sizes (mV)
Experiment 1				
QPS5-IBI2.5	51.0 ± 8.5	35.8 ± 4.0	–	0.39 ± 0.23
QPS5-IBI5	51.4 ± 9.3	38.0 ± 5.7	–	0.54 ± 0.30
QPS50-IBI2.5	52.4 ± 6.2	38.2 ± 3.6	–	0.78 ± 0.26
QPS50-IBI5	51.6 ± 9.4	40.0 ± 1.6	–	0.60 ± 0.33
Experiment 2				
QPS5-IBI5	54.4 ± 6.2	37.2 ± 3.3	–	0.62 ± 0.30
QPS5-IBI7.5	54.2 ± 8.1	39.2 ± 5.0	–	0.38 ± 0.12
QPS5-IBI10	53.4 ± 7.9	40.6 ± 5.0	–	0.50 ± 0.11
QPS50-IBI5	56.0 ± 3.2	38.8 ± 1.8	–	0.72 ± 0.25
QPS50-IBI7.5	52.6 ± 6.7	39.6 ± 5.2	–	0.56 ± 0.23
QPS50-IBI10	52.8 ± 8.0	38.4 ± 3.5	–	0.40 ± 0.11
Experiment 3				
QPS5-Mono	60.0 ± 13.6	42.2 ± 5.2	–	0.43 ± 0.13
QPS5-Bi	58.2 ± 8.1	43.2 ± 6.6	45.2 ± 4.4	0.39 ± 0.10
QPS50-Mono	57.3 ± 9.7	42.3 ± 5.6	–	0.42 ± 0.09
QPS50-Bi	60.0 ± 13.6	42.2 ± 5.2	45.3 ± 12.3	0.43 ± 0.13
Experiment 4				
QPS5	51.2 ± 10.6	37.8 ± 7.9	–	0.58 ± 0.26
QPS50	50.7 ± 9.1	38.2 ± 7.9	–	0.54 ± 0.24

indicating a less stable effect after QPS-IBI7.5 compared with QPS-IBI5. Indeed, one-way ANOVA on each condition also revealed significant main effect of TIME in QPS5-IBI5, QPS50-IBI5, and QPS50-IBI7.5, but not in QPS5-IBI7.5, QPS5-IBI10, and QPS50-IBI10 (Table 3). Both excitatory and inhibitory QPSs using IBI5 induced significant cortical excitability changes, those using IBI7.5 induced variable excitability changes and those using IBI10 induced no changes.

Experiment 3

To assess whether monophasic magnetic pulse configuration was more effective for inducing cortical excitability changes than biphasic pulses, we performed the experiments with monophasic and biphasic QPS. Fig. 2E and F shows the time course of each condition. Although both configuration types induced MEP changes depending on the ISI, they lasted approximately 25–30 min in the biphasic QPSs, which were shorter compared with monophasic QPSs. Three-way repeated measures ANOVA revealed significant ISI × TYPE × TIME interaction (Table 2), indicating that the time courses of each condition significantly differed. We found a significant difference between QPS-Mono and QPS-Bi after 30 min in both excitatory QPS5 and inhibitory QPS50 conditions (Bonferroni corrected, excitatory QPS5-Mono vs Bi; T30, $P = 0.038$; T45, $P = 0.015$: T60, $P = 0.003$: inhibitory QPS50-Mono vs Bi; T45, $P < 0.001$; T60, $P = 0.013$). In addition, separate one-way ANOVAs in each condition revealed shorter lasting changes of MEP in biphasic QPS (Table 3). The biphasic QPS induced shallower and shorter excitability changes than monophasic QPS.

Table 2
Results of three-way repeated measures ANOVAs.

Factor	df	Error	*F*	*P*
Experiment 1				
ISI	1	8	16.004	**0.004**
IBI	1	8	0.996	0.347
TIME	2.861	22.888	1.564	0.226
ISI × IBI	1	8	34.906	**<0.001**
ISI × TIME	2.971	23.768	0.686	0.568
IBI × TIME	2.861	22.888	0.232	0.865
ISI × IBI × TIME	2.971	23.768	2.293	0.104
Experiment 2				
ISI	1	12	51.099	**<0.001**
IBI	2	12	3.021	0.087
TIME	3.872	46.469	1.123	0.357
ISI × IBI	2	12	19.909	**<0.001**
ISI × TIME	3.438	41.255	1.229	0.313
IBI × TIME	3.872	46.469	0.510	0.838
ISI × IBI × TIME	6.876	41.255	0.948	0.480
Experiment 3				
ISI	1	10	24.567	**0.001**
TYPE	1	10	0.009	0.928
TIME	7	70	2.076	0.058
ISI × TYPE	1	10	2.358	0.156
ISI × TIME	7	70	3.122	**0.006**
TYPE × TIME	7	70	1.058	0.400
ISI × TYPE × TIME	7	70	4.522	**<0.001**

Bold indicates *P* value less than 0.05.

Experiment 4

In the final experiment, we investigated the variability of the QPS effect using the optimal stimulation parameters shown above for inducing plasticity. We had chosen QPS-IBI5 for 30 min for both excitatory and inhibitory QPSs, based on the findings obtained in experiments 1–3. Fig. 3A and B plots time courses of excitatory QPS5 (Fig. 3A) and inhibitory QPS50 (Fig. 3B) in all 35 subjects. Although there was a large variation in response between individuals, it appears that only a small number of subjects responded to QPS in the opposite way. Indeed, one-way ANOVA performed separately on excitatory QPS5 and inhibitory QPS50 data revealed a main effect of TIME in both excitatory QPS5 group ($F = 9.417$; df 6, 204; $P < 0.001$) and inhibitory QPS50 group ($F = 13.261$, df 6, 204; $P < 0.001$). Two-way repeated measures ANOVA on combined QPS-5/QPS-50 data showed a significant main effect of QPS ($F = 78.7$; df 1,68; $P < 0.001$) and TIME × QPS interaction ($F = 2.7$; Greenhouse–Geisser corrected df 3.3, 227.4; $P = 0.042$). Thus, on average, there was a potentiating effect of excitatory QPS5 and a depressive effect of inhibitory QPS50, consistent with original reports [13–15]. According to the criteria of responder and non-responder in previous studies [4,12], we calculated the average effect of QPS expressed as the mean of all post-QPS measures relative to baseline. Following excitatory QPS5, the mean of the average effect was 1.60 (SD = 0.57; 95% confidence interval 1.41–1.79); inhibitory QPS-50 decreased the response by 0.67 (SD = 0.22; 95% confidence intervals 0.60–0.74). Fig. 3C provides a simple summary of the response profile in this population in terms of whether the average effect at post-QPS period was greater or less than 1. Over 80% of participants increased their response after excitatory QPS-5 (86%) and decreased their response after inhibitory QPS-50 (94%). Although such a "dichotic" method to differentiate the plasticity response would be beneficial for a better understanding of the nature of NIBS plasticity, we have also attempted to evaluate the observed long-term effect based on the estimation of natural variation of MEP size. We calculated the expected variability based on MEPs after sham intervention (N = 12, all naïve subjects). The mean (standard deviation) of normalized MEP sizes for 30 min was 1.00 (0.12). Thus, the range of MEP changes under the sham condition was between 0.76 and 1.24 in normalized values. This value of normal range was nearly the same as a previously reported natural variation [12]. According to this criterion, it is possible to roughly differentiate "responders" (in whom expected responses (i.e., above 1.24 after excitatory QPS5 or below 0.76 after inhibitory QPS50) were obtained), "non-responders" (in whom MEP size lies within the above mentioned range, 0.76–1.24), and "opposite responders" (in whom opposite responses (i.e., below 0.76 after excitatory QPS5 or above 1.24 after inhibitory QPS50) were obtained). Fig. 3D is the response profile based on this classification. Although the responder rate is reduced in both excitatory QPS5 (from 86% to 80%) and inhibitory QPS50 (from 94% to 63%) interventions and the non-responder rate increased substantially compared with the above dichotic analysis, it should be noted that the opposite responder was only 3% in excitatory QPS5, and no opposite responder was found after inhibitory QPS50 (Fig. 3D).

Table 3
Results of one-way ANOVAs for each condition.

	df	Error	*F*	*P*	*P* values compared with baseline (Dunnett's test)									
					T5	T10	T15	T20	T25	T30	T40	T45	T50	T60
Experiment 1														
QPS5-IBI2.5	9	36	0.599	0.789										
QPS5-IBI5	9	36	2.936	**0.010**	0.348	0.121	0.162	**0.008**	**0.021**	**0.010**	**0.001**	–	**0.007**	**0.025**
QPS50-IBI2.5	9	36	1.016	0.444										
QPS50-IBI5	9	36	3.658	**0.002**	**0.017**	0.055	**0.001**	**0.001**	**0.001**	**0.011**	**0.003**	–	**0.004**	**0.015**
Experiment 2														
QPS5-IBI5	9	36	4.766	**0.001**	0.659	**0.034**	**0.018**	**0.002**	**0.004**	**0.001**	**0.002**	–	**0.007**	**0.016**
QPS5-IBI7.5	9	36	1.381	0.233										
QPS5-IBI10	9	36	0.803	0.616										
QPS50-IBI5	9	36	3.696	**0.002**	**0.003**	**0.002**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	–	**0.003**	**0.002**
QPS50-IBI7.5	9	36	3.060	**0.008**	**0.004**	**0.007**	0.731	**0.014**	**0.016**	**0.015**	**0.010**	–	**0.007**	0.065
QPS50-IBI10	9	36	0.597	0.790										
Experiment 3											–		–	
QPS5-Mono	8	40	2.887	**0.012**	0.198	0.246	0.050	**0.017**	**0.016**	**0.015**	–	0.052	–	**0.001**
QPS5-Bi	8	40	5.951	**0.001**	0.389	0.067	**0.010**	**0.001**	**0.002**	0.483	–	0.997	–	1.000
QPS50-Mono	8	40	8.068	**0.001**	**<0.001**	**0.002**	**<0.001**	**0.001**	**<0.001**	**<0.001**	–	**<0.001**	–	**<0.001**
QPS50-Bi	8	40	6.639	**0.001**	**<0.001**	**0.001**	**0.002**	**0.007**	0.783	0.573	–	0.999	–	0.765

Bold indicates *P*-value less than 0.05.

Discussion

The present results show that the response to the QPS protocol was mostly predictable and not highly variable at least in this cohort. This value suggests that QPS should be less variable than other plasticity protocols in which approximately 50% of the participants failed to respond in the "canonical" manner [2]. To the best of our knowledge, this is the first large-scale study of the variation in after-effects of QPS protocol in healthy volunteers. Before discussing this favorable outcome, it is important to consider which parameters are critical for the QPS protocol. Thus, we investigated which parameters of QPS might substantially affected QPS-induced plasticity.

Parameters of QPS

Experiment 1 revealed that although the total number of pulses was the same (i.e. 1440 pulses), the QPS at an IBI of 2.5 s (total duration of QPS, 15 min) did not induce any excitability changes, whereas the QPS at an IBI of 5 s induced long-lasting changes. In experiment 2, we showed that when the duration of the QPS was fixed at 30 min, plasticity was induced by QPS5/QPS50 at an IBI of 5 s (1440 pulses in total) and QPS50 at an IBI of 7.5 s (960 pulses), but neither by QPS5 at an IBI of 7.5 s (960 pulses) nor QPS5/QPS50 at an IBI of 10 s (720 pulses). Experiment 3 revealed that biphasic QPS induced shorter-lasting cortical excitability changes (up to 30 min after intervention) compared with monophasic QPS. These data indicated that similar to other NIBS protocols, no single but multiple parameters, including stimulus intensity, ISI, IBI, total number of pulses, duration of the whole intervention and pulse configuration, engaged in plasticity induction [19,20].

One unexpected result from experiment 1 was that the QPS at an IBI of 2.5 s did not induce any plasticity although the same total number of pulses (1440 pulses) of QPS at an IBI of 5 s induced significant plasticity. Previously, we showed that neither 15 min of QPS at an IBI of 5 s (i.e. 720 pulses in total) nor 20 min of QPS at an IBI of 5 s (i.e. 960 pulses in total) did induce any plasticity [13,14], although 30 min of QPS at an IBI of 5 s induced the plasticity. This finding indicated the dose dependency of QPS at an IBI of 5 s and 1440 pulses in total was optimal to induce plasticity by QPS. Thus, we first hypothesized that the total duration of monophasic QPS (i.e., 30 min) could be shortened to 15 min when an IBI of 2.5 s was used for monophasic QPS because the total number of pulses of this particular protocol was identical to conventional QPS (i.e., 1440 pulses). However, this finding was not observed in the present experiment: QPS at an IBI of 2.5 s for 15 min (i.e. 1440 pulses) did not induce any plasticity. The other puzzling result was that excitatory QPS5 at an IBI of 7.5 s did not induce any plasticity, while inhibitory QPS50 at the same IBI decreased MEP sizes significantly for approximately 50 min in experiment 2. These data indicated that there is a potential interaction among ISI, IBI, total duration of pulses, and stimulation time and that no single factor could determine the induction of plasticity. This finding was consistent with the previous seminal study performed by Huang et al., showing that different IBIs profoundly influenced plasticity induction, even with the same total number of pulses [21]. Such a complicated interaction was also confirmed by the modeling study [22]. Thus, it is likely that multiple parameters mutually interacted with each other for plasticity induction. This finding also indicated that it was necessary to investigate all of the potential combinations of parameters in order to determine optimal parameters of NIBS plasticity induction protocol. Unfortunately, it is impossible to draw a firm conclusion about the optimal IBI in the QPS protocol from the results of the present experiments as the design of our experiments did not disentangle the relative contribution of all parameters individually. It could be that the total duration of the intervention (30 min) is a critical factor to determine the final outcome most likely because a defined time may be necessary to initiate specific processes of plasticity induction, but this idea remains speculative and requires further studies for confirmation.

However, our results provide practically useful information because in all of the QPS experiments using an IBI of 5 s produced significant MEP changes depending on the ISI of four magnetic pulses, which indicated that the original QPS protocol might induce robust plasticity. None of the modified versions of QPS using other IBIs were optimal for inducing plasticity, when the total duration was fixed at 30 min. In addition, even when the total duration of application time was shortened from 30 min to 15 min using an IBI of 2.5 s, this did not produce any advantage in the efficacy of plasticity induction. Thus, although all possible conditions were not tested, the present results suggest that the original QPS protocol/parameters might be, at least at the moment, the most optimal for plasticity induction.

In experiment 3, we investigated whether monophasic QPS is better than biphasic QPS. These results showed that this was the case, as biphasic QPS after-effects had a much shorter duration than monophasic QPS. However, it is uncertain which neurons in the brain

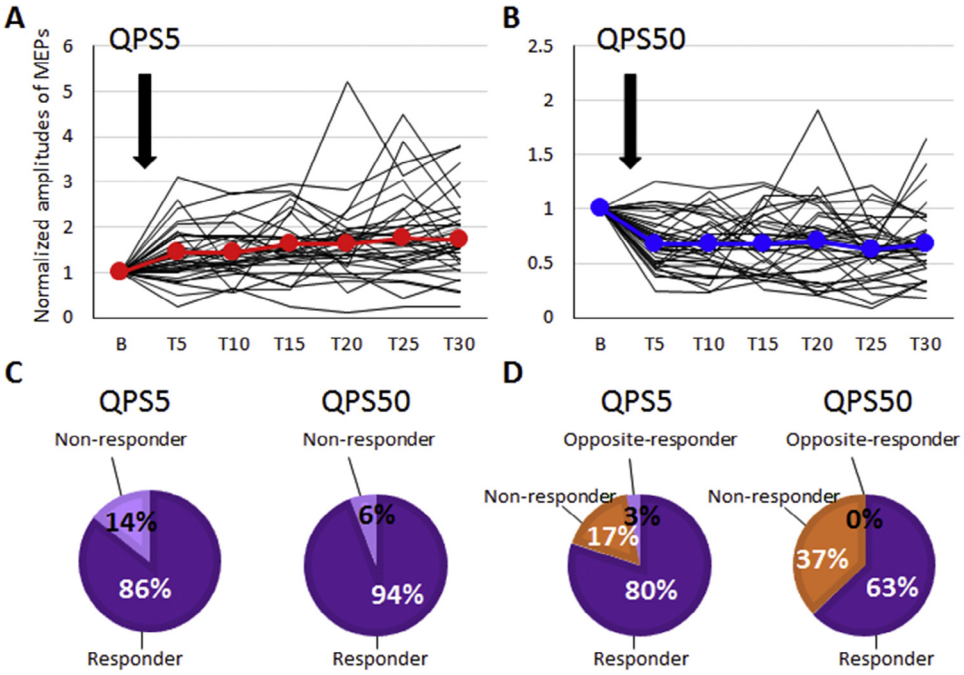

Figure 3. Time courses of excitatory QPS5 (A) and inhibitory QPS50 (B). The x-axis shows the time points and y-axis shows the normalized amplitude of MEPs to baseline (B). The thick black line and dot indicate the mean. (C, D) The percentage of responder, non-responder, and opposite responder is shown in each QPS session (see text).

are activated by magnetic stimuli, and we have no direct evidence for how monophasic and biphasic magnetic stimuli differ in the efficacy of neuronal excitation. However, according to epidural recording studies, it is likely that monophasic and biphasic pulses activate different sets of neurons in the motor cortex. A mixture of various interneurons can be activated by biphasic pulses, while monophasic pulses preferentially activate relatively homogenous population of neurons [23]. Accordingly, it has been assumed that the monophasic mode of repetitive TMS (rTMS) may have stronger after-effects than biphasic rTMS, as the activation of relatively uniform populations of interneurons by monophasic TMS would readily result in an effective summation of synaptic efficacy, while biphasic pulses may activate various interneurons, resulting in some cancellation of inhibitory and facilitatory effects with one another. Indeed, previous studies have clearly shown that monophasic rTMS is more effective than biphasic rTMS [17,24,25]. In keeping with these reports, we observed that monophasic QPS is capable of inducing longer-lasting after-effects than biphasic QPS. However, we did not adjust the stimulus intensity as we chose it relative to AMT, but this might be one confounding factor for interpretation.

Variability of QPS

Fig. 3 shows that the response to QPS protocols is relatively consistent across subjects. We chose to examine one particular, originally reported type of QPS with canonical choices of pulse configuration (monophasic), stimulus intensity (95% AMT), duration (30 min, i.e., 1440 pulses in total), ISI (5 or 50 ms), IBI (5 s), and target site (primary motor cortex), based on the findings obtained in experiments 1–3. These results were relatively consistent: after excitatory QPS5, approximately 80% of individuals showed facilitation, whereas after inhibitory QPS50 the proportions were approximately 10:90 (facilitation: inhibition). Grouping individuals according to whether the mean MEP amplitudes after QPS were larger or smaller than baseline was somewhat arbitrary. Due to this limitation, we also divided the individuals into "responder" (in whom expected responses (i.e. above 1.24 after excitatory QPS5 or below 0.76 after inhibitory QPS50) were obtained), "non-responder" (in whom normalized size lies within the above mentioned range, 0.76–1.24), and "opposite responder" (in whom opposite responses (i.e., below 0.76 after excitatory QPS5 or above 1.24 after inhibitory QPS50) were obtained) according to MEP variability evaluation after sham intervention. The responder rate was reduced in both excitatory QPS5 (80%) and inhibitory QPS50 (63%) and non-responder increased substantially compared with conventional classification. Unexpectedly, there were notably few opposite responders to excitatory QPS5 (3%) and no opposite responders to inhibitory QPS50. The main conclusion was that the after-effect of this type of QPS on corticospinal excitability was relatively consistent. Although we did not test the session-to-session variation within each person which might affect our results, recent studies have suggested that the intra-individual variability is lower than inter-individual variability [26]. Thus, it is likely that such intra-individual variability is also lower in QPS. However, this issue warrants further investigation.

Several determinants have been identified to explain the variability of NIBS plasticity protocols, such as age, gender, time of the day, physical activity, prior history of muscle activity, and genetics [11]. Furthermore, Hamada and colleagues have previously reported that the variability of recruitment of interneuron networks is another determinant for TBS and anodal TDCS [4,12], as they may also have a functional relevance in terms of motor learning [27]. However, we did not test these factors in relation to QPS variability as well as other potential sources of variability, such as ethnicity, direction of current flow, thickness of bone, which should be fully investigated in future studies.

Limitation

We are aware of the number of limitations in this study. First, the number of subjects in experiments 1–3 was small. Second, the study was performed in Japan, and thus, we cannot exclude the potential effect of ethnicity and/or genotype. Third, the duration of

effects of QPS with an IBI of 7.5 ms was not explored. Fourth, another factor to be considered would be “founder” effects, in which impressive effects for other NIBS paradigms have often been reported by founder labs.

Conclusions

The effects of QPS are variable with less than 20%–40% of individuals at maximum having poor or absent responses. QPS at an IBI of 5 s for 30 min would be the optimal protocol for inducing relatively constant long-term effects. However, we are uncertain whether less variable responses to QPS can be obtained even with measures other than corticospinal excitability, such as motor learning and clinical outcomes. Future studies are warranted to test these parameters in order to apply QPS as a treatment option for neurological diseases.

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